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Contraceptive Steroids Influence the Hemostatic Activation State in Healthy Men

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ABSTRACT: Hormonal contraception for men requires administration of testosterone and gestagens. The effects of a long-acting testosterone ester and 2 different progestins on hemostatic activation parameters were studied in relation to cardiovascular risk. In phase 1, 7 healthy men aged 28–38 years received a single intramuscular injection of 200 mg norethisterone-enanthate (NET-EN) on Day 0. Plasma samples were obtained on Days 0, 14, 41, and 84. In phase 2, 3 groups of 14 healthy men aged 18–45 years received four injections (every 6 weeks) of 1000 mg testosterone undecanoate (TU), plus daily oral placebo or daily oral levonorgestrel (LNG, 250 μg); or four injections (every 6 weeks) of NET-EN. Treatment lasted 24 weeks. Plasma samples were obtained at weeks 0, 16, 24, and 52. All samples were assayed for levels of coagulation factors VIIc, VIIa, XIIc, and XIIa; prothrombin fragment F1+2 (F1+2); antithrombin; plasmin-α_2-antiplasmin-complex (PAP); and fibrinogen. NET-EN alone led to a depletion of sexual hormones and a marked shift in hemostatic parameters with increasing levels of FXIIc, fibrinogen, antithrombin, and F1+2, whereas FVIIc and FVIIa levels decreased. PAP levels increased significantly. Opposite effects were seen in the TU/placebo group, with a significant down-regulation of fibrinolysis and the hemostatic turnover rate. Testosterone effects were attenuated by additional administration of gestagens. The effect of hormonal male contraception using long-acting testosterone esters with or without gestagens was significantly measurable within the hemostatic system. Down-regulation of the hemostatic system with testosterone alone may indicate an antithrombotic effect, whereas clinical consequences of an additional gestagen compound cannot be derived.

Key words: Cardiovascular risk, gestagens, hemostasis, hormonal male contraception, testosterone.

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Hormonal male contraception is an emerging reality. The suppression of gonadotropin production and, hence, spermatogenesis, can be achieved by administration of a combined regimen of testosterone and an additional gestagen (Nieschlag and Behre, 1998). The role of testosterone in atherosclerosis is still debatable. Epidemiological studies show plasma levels of testosterone to be associated with the incidence or presence of coronary heart disease (CHD; Philipps et al, 1994, Alexandersen et al, 1996; von Eckardstein, 1998). Abuse of androgens for anabolic purposes has been held responsible for myocardial infarction (Bagatell and Bremner, 1996; Sullivan et al, 1998), but to the contrary, administration of testosterone to men with CHD reduced clinical, electrocardiographic, and angiographic signs of coronary ischemia (Alexandersen et al, 1996; von Eckardstein, 1998; Rosano et al, 1999; Webb et al, 1999a,b). Confounding factors, especially in men with hypogonadism, include various metabolic disorders such as obesity, insulin resistance, dyslipidemia, and impaired fibrinolysis (von Eckardstein, 1998). This may explain findings of cross-sectional studies describing low total testosterone levels to be associated with cardiovascular risk factors (Barrett-Connor 1995; Simon et al, 1997).

Hemostatic parameters associated with cardiovascular risk include fibrinogen factors VII and XII (FVII and FXII, respectively), antithrombin, and prothrombin fragment F1+2 (F1+2) as substances involved in coagulation and plasminogen activator inhibitor type 1 (PAI-1) and plasmin-α_2-antiplasmin complex (PAP) as effectors of fibrinolysis. (PAP levels serve as a terminal, summarizing marker of the hemostatic system's turnover rate and are affected by PAI-1.)

Associations with androgen levels have been demonstrated in men for some of these parameters. Plasma levels of fibrinogen are inversely correlated with levels of endogenous and exogenous androgens in men (Glueck et al, 1993; Anderson et al, 1995; De Pergola et al, 1997). An inverse correlation is also seen between testosterone and PAI-1 as an antifibrinolytic parameter (Anderson et al, 1995; Sobel et al, 1995; Ferenchick et al, 1997; Adamkiewicz et al, 1999), although some authors have not been

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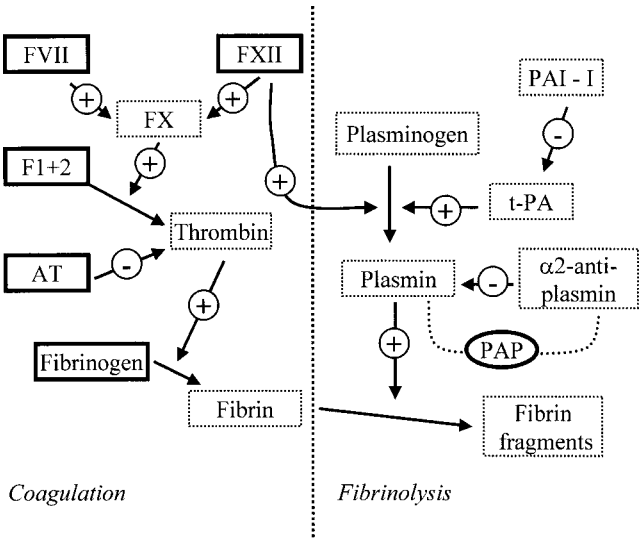

Figure 1. Simplified pattern of the hemostatic system to demonstrate the location of the investigated parameters related to cardiovascular risk. Note that major components have been left out (complete cellular phase, some coagulation factors, protein C, protein S, tissue activated fibrinolysis inhibitor, etc). Consult a textbook of hematology/hemostasis for complete information. The left panel shows the coagulation phase, the right panel shows fibrinolysis. Measured parameters are indicated with full frames, those not investigated are indicated with broken frames. Arrows with a plus or minus sign indicate the nature of influence wielded by the respective parameter (i.e., enhancing or inhibiting a process or substance). Precursor substances are connected to metabolites by arrows without signs.

able to describe a relationship with androgens (De Pergola et al, 1997; van Kesteren et al, 1998). Men with hypogonadism have low baseline fibrinolytic activity, which is accounted for by increased synthesis of PAI-1 (Winkler, 1996; Bennet et al, 1987; Zollner et al, 1997). The relationship between testosterone levels and FVIIc is seen inconsistently in cross-sectional studies (Philipps et al, 1993; Yang et al, 1993; De Pergola et al, 1997). In men, the effects of androgens on levels of the activated form of coagulation factor FVIIa, for which an association with cardiovascular risk seems to be more pronounced than for FVIIc (Ruddock and Meade, 1994; Meade et al, 1993; Junker et al, 1998), have not been described. Nor is there a description of possible influences of androgens on levels of PAP as a marker of plasmin generation and fibrinolytic balance, which is positively associated with atherothrombotic events (Sakkinen et al, 1999). In addition, potential effects by androgens on other factors associated with cardiovascular risk, such as F1+2 (Rugman et al, 1994; Carr, 2001), FXII (Ishii et al, 2000; Carr, 2001), and especially FXIIa (Kohler et al, 1998; Zito et al, 2000), have not been sufficiently investigated in men.

Clinically relevant side effects of additional gestagens have been demonstrated in women. The combination of estrogens and progesterone derivatives causes an increased risk for venous thrombosis (Quehenberger et al, 1996; Helmerhorst et al, 1997; Scarabin et al, 1997; Bellinger et al, 1998; Bonduki et al, 1998; Lidegaard et al, 1998; Vandenbroucke et al, 1999; Meijers et al, 2000; Middeldorp et al, 2000; Tans et al, 2000; Winkler et al, 2000). To our knowledge, the interrelationship of gestagens and testosterone, which may have a substantial effect on hemostatic parameters involved in cardiovascular risk and atherothrombosis, has not been described.

We performed this study with healthy men to investigate the effects of a steroid combination (testosterone undecanoate [TU] with the gestagen norethisterone-enanthate [NET-EN]) on activation parameters of the hemostatic system related to cardiovascular risk, and to determine these effects using different TU combinations with placebo or the gestagen, levonorgestrel (LNG). The location of the assessed parameters within the hemostatic system is displayed in Figure 1, which represents a simplified pattern of coagulation and fibrinolysis.

Materials and Methods

Study Design and Subjects

Subjects involved in this study were participating in other trials; results of those trials on contraceptive efficacy, which demonstrated a benefit of the TU/NET-EN regimen over TU/placebo and TU/LNG, have been published elsewhere (Kamischke et al, 2000a,b,c). This investigation presents further research. We assessed plasma samples from all participants in all trials. Results are presented in two phases; the first phase examines the effects of a single-dose pharmacokinetic agent involving NET-EN, and the second phase examines the influences of a longer-term contraceptive trial involving testosterone administration.

In a single-dose pharmacokinetic study (phase 1), 7 healthy

Table 1. *Values for C-reactive protein during the contraceptive trials**

C-Reactive Protein (mg/L)	Week 0	Week 16	Week 24	Week 52
TU/placebo	0.09 ± 0.02	0.14 ± 0.04	0.09 ± 0.08	0.12 ± 0.05
TU/levonorgestrel	0.17 ± 0.08	0.05 ± 0.01	0.19 ± 0.08	0.07 ± 0.02
TU/norethisterone-enanthate	0.09 ± 0.03	0.09 ± 0.03	0.08 ± 0.04	0.16 ± 0.05
	Day 0	Day 14	Day 41	Day 84
NET-EN single dose	0.19 ± 0.11	0.11 ± 0.05	0.08 ± 0.04	0.08 ± 0.04

* Data are given as means ± SEM. Changes from baseline were not significantly different (ANOVA for repeated measurements).

Table 2. *Testosterone levels during the single dose NET-EN study*[†]

Parameter	Day 0	Day 14	Day 41	Day 84
Total testosterone (nmol/L)	19.7 ± 3.0	3.2 ± 0.5***	15.1 ± 2.7	23.8 ± 2.1
Total estradiol (pmol/L)	57.2 ± 8.1	40.1 ± 2.7*	51.8 ± 3.3	60.9 ± 4.8
Free testosterone (pmol/L)	410 ± 45.9	85 ± 11.2***	365 ± 51.6	593 ± 47.6**

[†] Data are given as means ± SEM. Significant changes from baseline values are indicated with asterisks (*, **, ***, representing P values $< .05$, .01, and .001, respectively) according to nonparametric analysis for repeated measurements using the Friedman method (post hoc test according to Dunn).

white men, aged 28–38 years with no previous history of androgen preparation use, received a single intramuscular injection of 200 mg NET-EN at day 0 (Kamischke et al, 2000a).

The phase 2 study consisted of 3 hormonal regimens, each comprising 14 healthy white men aged 18–45 years with no previous history of androgen preparation use. Every volunteer received intramuscular injections of 1000 mg TU in study weeks 0, 6, 12, and 18 plus either daily oral placebo treatment, daily oral LNG (250 μg), or intramuscular injections of NET-EN (200 mg) in study weeks 0, 6, 12, and 18. The total treatment phase lasted 24 weeks, and oral medication was administered accordingly. The last follow-up visit was scheduled for week 52 (Kamischke et al, 2000b,c). Groups did not differ in age, body mass index, or smoking habits. Subjects did not experience severe infections or inflammatory disease throughout the trial (C-reactive protein [CRP] levels are provided in Table 1). The study was approved by the ethics committee of the university and the State Medical Board, Münster, Germany. All participants volunteered for the study and provided written informed consent.

Testosterone Undecanoate

This substance has not yet been introduced to the market. With an injection volume of 4 mL, the dose of 1000 mg TU in castor oil leads to maximal concentrations of 19.3 ± 2.1 nmol/L after 11.4 ± 1.5 days. The terminal half-life was determined as 33.9 ± 4.9 days. The optimal injection interval was set between 6 and 8 weeks (Nieschlag et al, 1999; Zitzmann and Nieschlag, 2000).

Levonorgestrel and Norethisterone-Enanthate

Both substances were supplied by Schering, Berlin, Germany. LNG is currently marketed by Schering as Microlut. NET-EN is marketed by Schering under the name of Noristerat.

Collection and Processing of Plasma Samples

All venous blood samples (9 parts blood in 1 part 0.13 M sodium citrate pH 7.8) were obtained after a fasting state in standardized conditions between 0800 and 1200 hours after a 30-minute rest. Plasma was separated at 800 × g on study days 0, 14, 41, and 84 (phase 1) or during study weeks 0, 16, 24, and 52 (phase 2). Samples were immediately stored at −80°C for a maximum of 12 months. The cooling device we used is equipped with an alarm system and provided temperature continuity during the time when samples were stored. Assays are not affected under these conditions. All assays were performed in one batch.

Laboratory Methods

Prothrombin time (normal range, 70%–130%), activated partial thromboplastin time (normal range, 24–35 seconds), FVIIc (normal range, 70%–120%), and FXIIc (normal range, 70%–150%) were measured using a Behring BCS coagulation analyzer and Thromborel S or Pathromtin SL, respectively, and a specific deficient plasma for FVIIc and FXIIc (all from Behring Diagnostics, Marburg, Germany). Using data provided by Clauss (1957), fibrinogen (normal range, 180–350 mg/dL) and antithrombin activity (normal range, 80%–120%) were determined using the BCS analyzer and Multifibren U (Behring Diagnostics) or a chromogenic substrate (Berichrom ATIII, Behring Diagnostics). FVIIa (normal range, 15.5–238.7 mU/mL) was also determined using the BCS analyzer and a clotting assay kit (Roche, Mannheim, Germany). PAP (normal range, 120–700 μg/L) was measured with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (WAK Chemie, Schwalbach, Germany) in the same method used to measure F1+2 (normal range, 0.4–1.1 nmol/L). Hematocrit (normal range, 42%–52%) and platelet count (normal range, 150–350 × 10^6/mL) were determined using the automated hematology analyzer Technicon H3 (Bayer, Fernwald, Germany). FXIIa was measured with an ELISA kit (Shield Diagnostic, Dundee, United Kingdom). The reference range was 1.0-3.0 ng/mL. C-reactive protein was determined nephelometrically on a BNII analyzer (Dade Bering, Schwalbach, Germany). The lower detection limit was 0.08 mg/L, the upper normal value was 0.5 mg/L.

For testosterone measurements, samples from the NET-EN single-dose study group, the TU/placebo group, and the TU/LNG group were measured using a commercial fluoroimmunoassay (Autodelfia, Wallac, Turku, Finland). Serum testosterone levels of the TU/NET-EN group were measured after changing to another commercial ELISA kit (DRG Instruments GmbH, Marburg, Germany). Levels of sex hormone binding globulin (SHBG) and estradiol were determined by highly specific time-resolved fluoroimmunoassays (Autodelfia). In our laboratory, the normal range for serum levels of total testosterone is 12–35 nmol/L, the upper normal limit for estradiol is 250 pmol/L, and the normal range for SHBG is 11–71 nmol/L. To maintain these parameters, our laboratory participates in a quality control scheme and regularly passes requirements. Levels of free testosterone were calculated from levels of SHBG and total serum testosterone according to the law of mass action, using 3.6 × 10^4 M^{-1} as the association constant of testosterone with albumin and 1 × 10^9 mol/L with SHBG. Calculation with this method yields highly reliable values of levels of free testosterone (Ver-

Table 3. *Hemostatic parameters during the single dose NET-EN study*[†]

Parameter	Day 0	Day 14	Day 41	Day 84
FVIIa (mU/mL)	68.1 ± 14.2	44.5 ± 9.1*	55.2 ± 5.1	65.8 ± 8.5
FVIIc (%)	97.5 ± 4.4	89.3 ± 3.7	95.4 ± 3.2	97.6 ± 5.4
Antithrombin (%)	98.6 ± 3.8	107.0 ± 3.1**	103.5 ± 2.6	99.9 ± 3.1
F1+2 (nmol/L)	0.49 ± 0.04	3.81 ± 1.64	3.46 ± 1.70	2.53 ± 1.30
FXIIa (ng/mL)	2.01 ± 0.17	2.02 ± 0.18	2.06 ± 0.20	2.06 ± 0.13
FXIIc (%)	110.1 ± 6.8	122.6 ± 5.6**	120.9 ± 4.6**	117.1 ± 6.1
Fibrinogen (mg/dL)	231.9 ± 14.1	286.2 ± 13.9*	262.6 ± 13.7	261.2 ± 11.6
PAP (μg/L)	184.0 ± 23.3	479.5 ± 66.6***	352.9 ± 28.2**	248.8 ± 42.6

[†] Data are given as means ± SEM. Significant changes from baseline values are indicated with asterisks (*, **, ***, representing *P* values < .05, .01, and .001, respectively) according to nonparametric analysis for repeated measurements using the Friedman test (post hoc test according to Dunn).

meleulen et al, 1999). The laboratory staff was unaware of the patients' treatment schedules.

Statistical Analysis

All variables were checked for normal distribution by the Kolmogorov-Smirnov one-sample test for goodness of fit by applying the modified calculation of statistical significance according to the Lilliefors test (stricter criteria). Due the small number (n = 7) of subjects in the NET-EN single-dose group, putative changes from baseline in this part of the study were calculated using the Friedman nonparametric test for repeated measurements (ie, the Dunn post hoc test for comparison with baseline values). To compare differences between the groups of the second, larger phase of the study, instead of using analysis of covariance on changes from baseline (by incorporating baseline values as covariates), values were transformed within each group to homogenous, dimensionless baseline values of 100. Thus, without impairing statistical power, variations between study groups were evaluated by two-way analysis of variance (ANOVA) because all parameters followed a gaussian distribution. Because this is not always the case for F1+2 and PAP, these values were logarithmically transformed for analysis as well (different results were not observed). Changes from baseline for each specific group were calculated by ANOVA for repeated measurements; overall, when $P < .05$, the Dunnett post hoc test for changes from baseline values was performed. The influence of changing levels of sexual hormones on the described parameters was evaluated with time-series analyses using cross-correlations. Fibrinogen is an inflammation marker and was positively associated with CRP. Aside from analysis of uncorrected values of fibrinogen levels and in order to exclude influences from minor viral infections, which occurred in some subjects, fibrinogen levels were adjusted for CRP to properly determine the effect of the study drugs (also see CRP levels in Table 1).

Computations were performed using a statistical software package from SPSS (Chicago, Ill; release 9.0.1.). Unless otherwise stated, results are given as means ± SEM in tables and figures. Two-sided *P* values < .05 were considered significant. For ANOVA results, levels of statistical significance are followed by asterisks (*,**,***) representing *P* values less than .05, .01, and .001, respectively.

Results

Phase 1

A highly significant suppression of serum levels of total and free testosterone was achieved during the NET-EN single-dose study, and estradiol levels decreased as well (Table 2). Changes in hemostatic parameters are given in Table 3. Levels of FVIIa decreased significantly, whereas those of antithrombin, FXIIc, fibrinogen, and especially PAP, increased significantly (results of the latter are displayed in Figure 2). This indicates a major increment in the hemostatic turnover rate.

Phase 2

Total testosterone levels increased with high significance in the TU/placebo group; changes in comparison to baseline were not significant in either gestagen treatment group. Due to a significant decrease in SHBG levels, concentrations of free testosterone increased in both gestagen treatment groups; there also was high significance in the TU/placebo treatment group (Table 4). In the TU/placebo group, significantly decreasing levels of FXIIc and PAP were observed, and antithrombin levels decreased significantly as well. The TU/LNG group demonstrated significantly decreasing levels of FVIIa and FXIIc; in the TU/NET-EN group, significantly decreasing levels of FVIIc and fibrinogen were observed (Table 5, Figure 3). Significant differences with two-way ANOVA between the TU/gestagen treatment group and the TU/placebo treatment group are displayed in Table 6. For the hemostatic turnover rate, a highly significant difference between the TU/gestagen group and the TU/placebo group was observed; a significant down-regulation occurred in the latter group, whereas stable conditions were maintained in the groups that received an additional gestagen.

Other Parameters

Other parameters were assessed in samples of the contraceptive trial; all values remained within normal ranges for

Table 4. *Testosterone and estradiol levels during the contraceptive trials*†

Parameter	Week 0	Week 16	Week 24	Week 52
Total testosterone (nmol/mL)				
TU/placebo	26.4 ± 2.2	32.2 ± 1.3**	28.1 ± 1.7	27.0 ± 1.8
TU/levonorgestrel	25.5 ± 1.4	26.2 ± 1.5	23.6 ± 1.3	23.8 ± 1.1
TU/norethisterone-enanthate	19.4 ± 1.9	21.1 ± 1.6	21.9 ± 2.0	16.9 ± 1.1
Total estradiol (pmol/L)				
TU/placebo	78.3 ± 6.8	90.43 ± 5.5	82.9 ± 7.3	76.4 ± 4.3
TU/levonorgestrel	79.8 ± 3.9	96.1 ± 7.2**	83.4 ± 5.4	65.9 ± 3.4*
TU/norethisterone-enanthate	66.6 ± 5.1	79.3 ± 5.4	75.9 ± 5.6	59.0 ± 3.7
Free testosterone (pmol/L)				
TU/placebo	584 ± 43.4	788 ± 40.7**	687 ± 41.1	617 ± 30.7
TU/levonorgestrel	578 ± 33.2	808 ± 57.2**	713 ± 48.5*	544 ± 23.6
TU/norethisterone-enanthate	429 ± 60.0	610 ± 61.6**	594 ± 69.1**	372 ± 30.3

† Data are given as mean ± standard error of the mean (SEM). Significance levels are given as asterisks (*, **, ***, representing P values < 0.05, 0.01, 0.001, respectively) for comparison to baseline values (post-hoc test after ANOVA for repeated measurements).

all groups. A significant increase in platelet count was observed for the TU/LNG group. Hematocrit levels increased significantly in both gestagen treatment groups but remained unchanged in the TU/placebo group (Table 7). Coagulation times remained within normal limits and were not significantly altered.

Relationship of Hemostatic Parameters to Sexual Hormone Levels

For results in the TU/placebo group, time series–dependent cross-correlations revealed that serum levels of estradiol were positively associated with FVIIa levels (r = .32, P = .019), although this model accounts for only 9.8% of the variance. Serum levels of testosterone predicted negative levels of FXIIc with high significance (r = −.41, P = .002); this model accounts for 16.6% of the variance. This observation, albeit weaker, was discernable for FXIIa as well. Serum levels of total testosterone also predicted negative antithrombin levels with high significance (r = −.41, P = .001). Testosterone levels accounted for 17.2% of the variance in antithrombin levels.

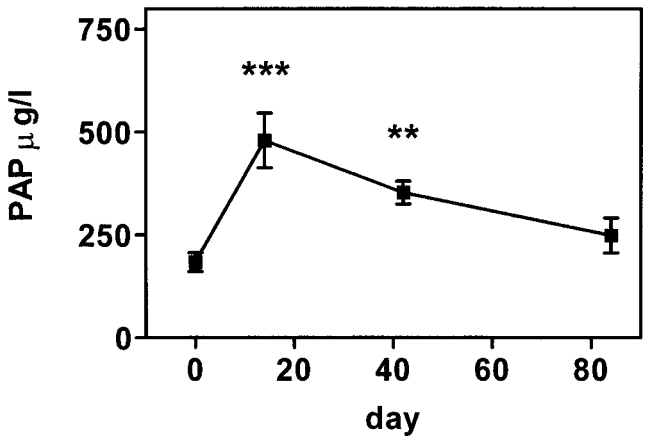

Figure 2. PAP levels from phase 1 (single-dose pharmacokinetic study with NET-EN) are displayed as means ± SEM. Results of nonparametric analysis for repeated measurements (the Friedman test) are displayed only as significant results of post hoc tests (the Dunn test) at specific time points (ie, change from baseline) as **, or *** (representing $P < .01$, and .001, respectively).

Discussion

Both testosterone depletion and external androgen administration exerted significant, opposite effects on hemostatic parameters related to atherothrombosis. Single-dose administration of NET-EN led to a marked depletion of testosterone to levels associated with hypogonadism, whereas during treatment with TU/placebo, testosterone levels increased significantly. Although androgen depletion was associated with a marked increase in the hemostatic system's turnover rate, the opposite was the case with increasing testosterone levels. It can be debated that effects of single-dose NET-EN were also due to direct progestagenic effects or estrogen depletion, although changes in the latter parameter were small compared to those of androgen depletion.

PAP levels as the terminal marker of fibrinolysis were affected by both testosterone depletion and testosterone administration, but because levels of direct products of fibrin formation, PAI-1, or the effects of factor XI (Bouma and Meijers, 2000) or thrombin-activated fibrinolysis inhibitor, which is not directly associated with cardiovascular risk factors (Juhan-Vague et al, 2000a), were not assessed during this study, it remains to be speculated how this effect was generated. Cytokine-induced production of FXII, which consequently activates the fibrinolytic system via the urokinase-plasminogen pathway, is a possible mediator of this effect, as levels of FXIIc and FXIIa were significantly positively correlated with PAP levels and showed a negative association with those of testosterone. An activation of fibrinolysis by decreased levels

Table 5. *Hemostatic parameters during the contraceptive trials*[†]

Parameter	Week 0	Week 16	Week 24	Week 52
FVIIa				
TU/placebo	100.0 ± 26.3	115.8 ± 32.5	123.1 ± 31.6	104.6 ± 28.9
TU/levonorgestrel	100.0 ± 23.8	54.2 ± 6.5**	51.9 ± 6.8**	96.6 ± 23.9
TU/norethisterone-enanthate	100.0 ± 13.2	83.1 ± 12.8	83.6 ± 11.5	113.4 ± 17.6
FVIIc				
TU/placebo	100.0 ± 9.4	88.2 ± 8.7	92.7 ± 10.3	99.1 ± 10.6
TU/levonorgestrel	100.0 ± 6.7	94.5 ± 7.2	92.2 ± 3.1	98.7 ± 6.8
TU/norethisterone-enanthate	100.0 ± 4.3	95.8 ± 4.6	93.8 ± 4.0**	101.9 ± 4.2
Antithrombin				
TU/placebo	100.0 ± 2.0	96.5 ± 1.7	92.1 ± 1.9**	94.3 ± 2.0*
TU/levonorgestrel	100.0 ± 2.7	96.6 ± 3.7	98.9 ± 2.1	91.8 ± 4.1
TU/norethisterone-enanthate	100.0 ± 1.9	96.9 ± 1.8	95.9 ± 1.5	101.0 ± 1.8
F1+2				
TU/placebo	100.0 ± 31.2	95.3 ± 27.4	107.6 ± 32.9	104.6 ± 32.3
TU/levonorgestrel	100.0 ± 19.8	133.8 ± 48.3	112.8 ± 48.5	161.8 ± 49.8
TU/norethisterone-enanthate	100.0 ± 8.1	119.9 ± 11.3	137.9 ± 31.2	108.2 ± 11.7
FXIIa				
TU/placebo	100.0 ± 30.8	71.4 ± 8.6	80.2 ± 11.1	81.1 ± 12.7
TU/levonorgestrel	100.0 ± 6.9	99.9 ± 6.9	108.3 ± 9.9	103.8 ± 9.1
TU/norethisterone-enanthate	100.0 ± 7.7	94.2 ± 6.8	90.6 ± 6.6	100.3 ± 6.9
FXIIc				
TU/placebo	100.0 ± 6.8	81.6 ± 7.0**	85.5 ± 8.3*	95.5 ± 6.8
TU/levonorgestrel	100.0 ± 2.3	89.9 ± 4.0*	87.3 ± 4.0**	93.2 ± 3.6
TU/norethisterone-enanthate	100.0 ± 6.3	97.4 ± 5.8	97.7 ± 5.6	105.2 ± 5.5
Fibrinogen				
TU/placebo	100.0 ± 7.8	95.6 ± 4.4	89.9 ± 6.2	89.9 ± 5.9
TU/levonorgestrel	100.0 ± 4.1	104.6 ± 4.2	104.8 ± 5.9	99.2 ± 5.7
TU/norethisterone-enanthate	100.0 ± 3.6	93.2 ± 2.5*	92.5 ± 2.7*	98.6 ± 3.4
PAP				
TU/placebo	100.0 ± 27.4	72.2 ± 17.3	49.1 ± 11.9**	49.6 ± 8.2**
TU/levonorgestrel	100.0 ± 15.4	120.3 ± 21.6	119.8 ± 28.6	80.3 ± 13.5
TU/norethisterone-enanthate	100.0 ± 9.7	103.0 ± 12.0	92.2 ± 6.5	110.7 ± 9.0

[†] Data are given as means ± SEM after homogeneous transformation to a dimensionless baseline value of 100 to allow two-way ANOVA (see "*Materials and Methods*" and Table 3). Significant changes from baseline values are indicated with asterisks (* and **, representing P values $< .05$ and .01, respectively) for comparison to baseline values (post hoc test according to Dunnett after ANOVA for repeated measurements).

of PAI-1 is unlikely because this protein is known to be increased in hypogonadism (Bennet et al, 1987; Winkler, 1996).

According to our observations, the additional administration of progestins can partly influence these effects. Significant differences were seen for FVIIa, FXIIa, FXIIc, antithrombin, fibrinogen, and especially PAP. The gestagens seem to mitigate testosterone-induced influence on the hemostatic system. Both LNG (which is 18-methyl-NET) and NET-EN have androgenic activity, whereas their proper antiandrogenic activity is scarce or even debatable (Lobo, 1988; Neumann et al, 1988; Deckers et al, 2000). Antiandrogenic effects could rather be exerted by their 5α-reduced metabolites, which possess high androgen receptor binding capacities but diminished androgenic activity (Perez-Palacios et al, 1992). Compared to NET-EN, LNG has a higher affinity to the androgen receptor and exerts stronger androgenic effects. This also applies to the progesterone receptor; the affinity of LNG to the progesterone receptor is about 2–3 times stronger than that of NET-EN and may explain the differential influences (Bergink et al, 1983; Hoppen and Hammann 1987; Lobo, 1988; Perez-Palacios et al, 1992; Deckers et al, 2000). Both substances do not bind to the estrogen receptor in a measurable amount (Bergink et al, 1983; Hoppen and Hammann, 1987). It must be considered that gestagens can bind to SHBG and displace testosterone, and can decrease levels of SHBG, thus increasing free testosterone levels (Nilsson and von Schoultz, 1989; Van der Vange et al, 1990; Raudaskoski et al, 1998).

Changes in hemostatic parameters must be discussed from the perspective of lipid parameters as well. During the NET-EN single-dose study, levels of high-density lipoprotein cholesterol (HDL-C) and lipoprotein(a) de-

Table 6. *Comparison between gestagen and placebo treated groups*[†]

Parameter	TU/LNG vs. TU/Placebo	TU/NET-EN vs. TU/Placebo	TU/NET-EN vs. TU/LNG
FVIIa	↓		
FVIIc			
Antithrombin		↑	
F1+2			
FXIIa	↑		
FXIIc		↑	*
Fibrinogen	↑		*
PAP	↑↑	↑↑	

[†] Comparison to TU/placebo treated group: significant differences indicated by arrows (↑, ↑↑, and ↑↑↑, symbolizing that overall values were higher for the gestagen-treated group with $P < .05$, $< .01$, and $< .001$, respectively in two-way ANOVA of values displayed in Table 4; ↓ symbolizing significantly lower values for the gestagen-treated group; * indicating significant differences between LNG and NET-EN treatment groups.

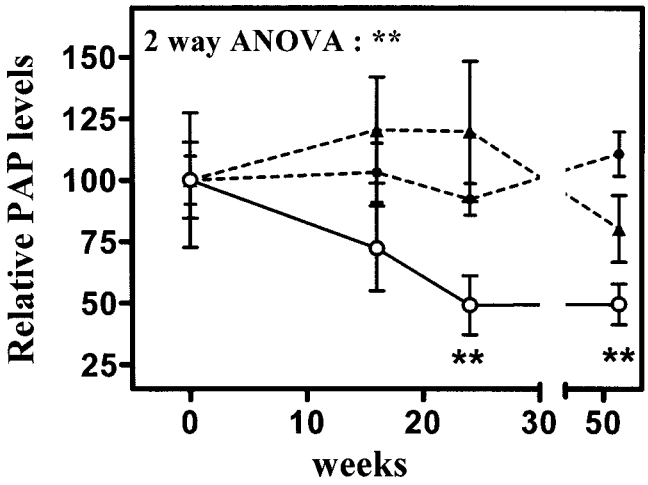

Figure 3. PAP levels from phase 2 (contraceptive trial) are displayed as means ± SEM; TU/placebo (○, full line), TU/LNG (●, dashed line), and TU/NET-EN (▲, dashed line). Results of two-way ANOVA (difference of curves) are shown with asterisks (* and **, representing $P < .05$ and .01 respectively). For TU/placebo, significant changes from baseline are displayed as asterisks according to ANOVA for repeated measurements.

creased significantly, whereas levels of low-density lipoprotein cholesterol (LDL-C) increased (Kamischke et al, 2000a). During the contraceptive trials, LDL-C levels increased significantly in the TU/NET-EN group, whereas levels in the TU/placebo and TU/LNG groups decreased slightly, but not significantly. HDL-C levels decreased significantly in all 3 groups. Levels of antifibrinolytic lipoprotein(a) were lower in both groups that received gestagens but remained unchanged in the TU/placebo group (Kamischke et al, 2000b,c). Decreasing levels of PAP can therefore not be attributed to changes in lipoprotein(a).

Testosterone given as single substance in the form of a long-acting ester leads to a lower turnover rate in the hemostatic system, which is mirrored by decreasing PAP levels. PAP reflects reactive fibrinolysis and is associated with subclinical atherosclerosis, especially in the presence of concomitantly increased levels of fibrinogen (Stein et al, 1997; Sakkinen et al, 1999). In persons with subclinical insulin resistance and obesity, however, hypofibrinolysis due to increased levels of PAI-1 and consequent depressed plasmin generation may enhance the progression of atherosclerosis (Meade et al, 1993; Juhan-Vague et al, 2000b). Thus, the observed effects of testosterone need to be regarded differentially for cardiovascular risk and atherothrombosis; the effects may benefit healthy persons, but they could have an adverse influence in those afflicted with insulin resistance.

In conclusion, single-dose NET-EN or TU alone exhibit prothrombotic and antithrombotic effects, respectively, which may exert clinical adverse or beneficial effects. Hormonal male contraception by a combined regimen of long-acting testosterone esters and progestins shows, in summary, few effects within the hemostatic system of healthy men, and absolute values remained within normal ranges. Nevertheless, testosterone and gestagen effects are detectable and demonstrate the necessity for further long-term investigations to determine a clinical meaningfulness.

Table 7. *Values for platelets and hematocrit during the contraceptive trials*[†]

Parameter (adjusted to a baseline value of 100)	Week 0	Week 16	Week 24	Week 52
Platelets				
TU/placebo	100.0 ± 4.1	101.8 ± 4.5	103.8 ± 5.5	102.0 ± 5.1
TU/levonorgestrel	100.0 ± 3.7	104.9 ± 3.1	107.9 ± 3.7*	97.2 ± 3.4
TU/norethisterone-enanthate	100.0 ± 6.1	102.3 ± 5.6	103.2 ± 5.5	98.3 ± 4.4
Hematocrit				
TU/placebo	100.0 ± 1.5	102.2 ± 2.1	102.4 ± 1.3	99.2 ± 1.1
TU/levonorgestrel	100.0 ± 1.3	104.6 ± 1.7*	104.2 ± 1.5*	96.4 ± 0.9
TU/norethisterone-enanthate	100.0 ± 1.5	107.9 ± 1.2**	108.4 ± 1.4**	100.3 ± 1.4

[†] Data are given as means ± SEM. Significance levels are shown with asterisks (* and **, representing P values $< .05$ and .01, respectively) for comparison to baseline values (post hoc test after ANOVA for repeated measurements). Values were adjusted for comparison between groups. All values remained within normal ranges.

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